

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

The following paragraph was inserted after the title at page 1 of the specification:

--Related Applications

This application claims priority to PCT International Application PCT/US98/18432 filed on September 4, 1998 which claims priority to U.S. Provisional Application 60/057,941 filed on September 5, 1997, the contents of which are expressly incorporated herein by reference.--

The paragraph beginning at page 1, line 12, was replaced by the following:

--Septic patients usually die as a result of poor tissue perfusion and injury followed by multiple organ failure. It is well recognized that many of the responses that occur during septic shock are initiated by bacterial endotoxin, a glycolipid antigen present on the surface of gram negative bacteria. This endotoxin (also referred to herein as lipopolysacchride or LPS) is released upon the death or multiplication of the bacteria and is known to activate monocytes/macrophages or endothelial cells causing them to produce various mediator molecules such as toxic oxygen radicals, hydrogen peroxide, tumor neurosis factor-alpha (TNF α), and interleukin (IL-1, IL-6, and IL-8). ~~Theses~~ These cellular and humoral inflammatory mediators evoke septic shock with symptoms ranging from chills and fever to circulatory failure, multiorgan failure, and death.--

The paragraph beginning at page 1, line 22, was replaced by the following:

--The impact of sepsis is particularly devastating to patients with compromised cardiac and hepatic function and to immunocompromised patients. Patients at high risk are elderly, ~~chemotheapy~~ chemotherapy patients and those requiring surgery or invasive instrumentation. The current therapy of antibiotics and hemodynamic support has not proven to be successful. An improved method for treating or preventing septic shock would be of great value.--

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The paragraph beginning at page 2, line 30, was replaced by the following:

--Accordingly, this invention provides compositions and methods for treating or preventing septic shock in a subject at risk of developing septic shock. The method comprises administering an effective amount of an agent which binds G protein such that septic shock is treated or prevented in the subject. The agents which bind G protein are useful for both ~~prophylactic~~ prophylactic and/or therapeutic treatments of septic shock.--

The paragraph beginning at page 3, line 24, was replaced by the following:

--Figure 4 shows that mastoparan only inhibits CD14-dependent LPS-induced signal transduction in U373-CD14 ~~transfected~~ transfected cells.--

The paragraph beginning at page 5, line 24, was replaced by the following:

--The term "administering" is intended to include routes of administration which allow the agent to perform its intended function of treating or preventing septic shock by binding to G protein. Examples of routes of administration which can be used include ~~parental~~ parenteral injection (e.g., subcutaneous, intravenous, and intramuscular), intraperitoneal injection, oral, inhalation, and transdermal. The injection can be bolus injections or can be continuous infusion. Depending on the route of administration, the agent can be coated with or disposed in a selected material to protect it from natural conditions which may detrimentally ~~effect~~ affect its ability to perform its intended function. When the agent is a peptide, such as mastoparan or analog thereof, the peptide can be modified at one or more of its termini to protect the peptide from degradation. Methods of protecting peptides from degradation are disclosed in U.S. Patent No. 5,589,568 which is incorporated herein by reference. The agent can be administered with other agents and/or with a pharmaceutically acceptable carrier. Further, the agent can be administered as a mixture of agents which bind G proteins, which also can be coadministered with a pharmaceutically acceptable carrier. The agent can be administered prior to the onset of septic shock or after the onset of septic shock.--

The paragraph beginning at page 6, line 17, was replaced by the following:

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-- The regimen of administration can affect what constitutes an effective amount. G protein binding agents can be administered to the subject either prior to or after the onset of septic shock. Further, several divided dosages, as well as staggered dosages, can be administered daily or sequentially, or the dose can be continuously infused or can be a bolus injection. Further, the dosages of the G protein binding agent(s) can be proportionally increased or ~~decrease~~ decreased as indicated by the exigencies of the therapeutic or prophylactic situation.--

The paragraph beginning at page 9, line 15, was replaced by the following:

-- **Isolation of human PBMC and monocytes.**

Freshly isolated human peripheral blood mononuclear cells (PBMC) and monocytes were obtained from leukopaks (discarded leukocyte from platelet donations). The cells were fractionated on FICOLL-HYPAQUE™ gradients, washed, treated with tris-buffered NH₄Cl to eliminate RBCs and washed to obtain PMBCs. Monocytes were obtained by depleting the PBMCs of T cells and NK cells by negative selection asking standard techniques. T cells and NK cells were removed by treatment with anti-CD3 and anti-CD2 monoclonal antibodies followed by goat anti-mouse Ig conjugated magnetic beads at a 10:1 bead:cell ratio. The monocyte preparations were at least 80-85% monocytes, as determined by anti-CD14 staining and forward and side light scatter analysis using a FACScan (Becton-Dickenson, Elmhurst, IL). Less than 2% of the contaminating cells in the monocyte preparation were T cells and no NK cells could be detected. Monocytes were maintained in Ham's F-12 10% FCS, L-Glutamine and ~~penacillin~~ penicillin/streptomycin at 37°C in 5% CO₂.--

The paragraph beginning at page 11, line 16, was replaced by the following:

-- **Lethal endotoxin shock**

Wistar rats (200 g) were obtained from Charles River Laboratories. Rats were treated with 3mg/kg mastoparan by intravenous injection in the tail vein, immediately followed by 15 mg/kg lead acetate and 5 µg/kg LPS 0111:B4 intravenously. Mortality was assessed up to 96 hours following LPS treatment. Mortality frequency was compared by Fisher exact test and statistical analysis was performed using Yates corrected Chi square test.--

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The paragraph beginning at page 11, line 24, was replaced by the following:

-- **Example 1. Association of CD14 with G Proteins Following LPS Stimulation**

To elucidate the mechanism of LPS-induced signal transduction mediated through CD14, CD14 was immunoprecipitated from freshly isolated human monocytes and *in vitro* kinase assays performed to ~~asses~~ assess the association of CD14 with phosphorylated proteins:--

In the Claims:

Claims 1 and 11 were amended as follows:

1. **(Amended)** A method for treating or preventing septic shock in a subject comprising, administering to the subject an effective amount of an agent which binds G protein, to thereby inhibit the interaction of said G protein with CD14, such that septic shock in the subject is treated or prevented.

11. **(Amended)** A composition for treating or preventing septic shock in a subject comprising an effective amount of an agent which binds G protein ~~to treat or prevent septic shock~~, to thereby inhibit the interaction of said G protein and CD14, such that septic shock in the subject is treated or prevented.

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APPENDIX A

1. **(Amended)** A method for treating or preventing septic shock in a subject comprising administering to the subject an effective amount of an agent which binds G protein, to thereby inhibit the interaction of said G protein and CD14, such that septic shock in the subject is treated or prevented.
2. The method of claim 1, wherein the agent binds G α subunit.
3. The method of claim 1, wherein the agent is a cell permeable agent.
4. The method of claim 3, wherein the agent is a peptide.
5. The method of claim 4, wherein the peptide is mastoparan or an analog thereof.
6. The method of claim 1, wherein the septic shock is endotoxic shock.
7. The method of claim 6, wherein the endotoxic shock is induced by gram negative bacteria.
8. The method of claim 1, wherein the endotoxic shock is induced by gram positive bacteria.
9. The method of claim 1, wherein the septic shock is LPS-induced shock.
10. The method of claim 1, further comprising administering an antibiotic to the subject.
11. **(Amended)** A composition for treating or preventing septic shock in a subject comprising an effective amount of an agent which binds G protein to thereby inhibit the interaction of said G protein and CD14, such that septic shock in the subject is treated or prevented.

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12. The composition of claim 11, wherein the agent binds $G\alpha$ subunit.
13. The composition of claim 11, further comprising a pharmaceutically acceptable carrier.
14. The composition of claim 11, wherein the agent is a cell permeable agent.
15. The composition of claim 14, wherein the cell permeable agent is a peptide.
16. The composition of claim 15, wherein the peptide is mastoparan or an analog thereof.
17. The composition of claim 11, further comprising an antibiotic.

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